

# REPORT DOCUMENTATION PAGE

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13. Abstract INTRODUCTION: Because of concerns for anthropogenic acoustic impacts in the oceans, much of the focus of the ONR marine program has been on expanding our knowledge of hearing in marine mammals and particularly understanding hearing in species likely to be low frequency sensitive. For practical reasons, most marine mammal hearing research is on audiometry for captive odontocetes and pinnipeds and on playback responses and modeling of hearing in mysticetes, which are mid to shallow range divers. However, another group of marine mammals, the deep divers, also needs to be assessed, particularly because these are ears that may have significant pressure adaptations. Prior to this study, pressure and auditory adaptations of deep divers had not been systematically investigated. Beaked whales are a particular focus in this study because they are ubiquitous but poorly understood. Beaked whales are large, pelagic, and elusive. They rarely strand. Consequently our knowledge of them is limited primarily to off-shore sitings, surface observations, and gross surface measurements of poorly preserved animals. For some species, well-preserved material is available but has not been dissected because of its rarity. In the last several years, by-catch specimens have dwindled because fisheries that impacted beaked whales are progressively being closed. Consequently, this study was opportunistic and brief; i.e., it was a small focused, exploratory study that utilized a narrow window of opportunity to obtain material for the rarest of the deep-diving species, the beaked whales.		
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Material obtained from by-catch and strandings was studied using high resolution non-invasive imaging and, for a few specimens, conventional histology. The study was designed to gather preliminary data to test two interdependent hypotheses: that deep divers have more robust barotrauma preventative mechanisms than shallower species and that they also have mechanisms to preserve hearing at depth.

**OBJECTIVES:**

- 1) To understand how ears in deep-diving marine mammals are structured to prevent barotrauma;
- 2) To determine whether dive-related adaptations affect hearing.

**RATIONALE:**

Among cetaceans, the beaked whales (Ziphiidae) and sperm whales (Physeteridae) are believed to be the deepest regular divers. We lack even basic descriptions of the ear and throat anatomy for virtually all beaked whales and most physeterids. We have no hearing data on any member of these two groups and there are no clear vocalization data for beaked whales. There is more information on the physiology of the deep diving pinnipeds, but none has been studied explicitly in terms of ear-pressure adaptations. Consequently, we have no knowledge of what auditory structural adaptations these animals evolved to endure bathypelagic and rapidly changing pressures nor do we understand whether their hearing alters with depth.

Deep-diving - even natural deep-diving without auxiliary supports - entails some hazard from the extremes of pressure encountered. Barotrauma is pressure induced damage. It can and does occur in free dives and generally impacts the lung, larynx, or ear regions. In the ear region, there is a particular hazard related to the spontaneous closure and inability to reventilate the Eustachian tube. In this case, the diver/animal cannot equilibrate pressures between the ambient and adjacent air-filled cavities, including the middle ear, resulting in ear membrane ruptures. Humans who sustain auditory barotrauma become nauseated and disoriented and can lose hearing permanently. Clearly, it is unlikely that marine mammals are as fragile as human divers. Considering the demonstrated importance of hearing for marine mammals coupled with their known diving capacities, it is reasonable to expect that ears in deep-diving marine mammals are model systems for successful deep-diving adaptations that also preserve hearing.

Understanding how marine mammal diver ears are structured can help answer questions that are important for ONR and the Low Frequency Sound Initiative. First, understanding how deep-diving species prevent barotrauma is fundamentally important for improving diver safety. Second, deep-diving whales are among the largest odontocetes, and if these diving species follow normal trends in hearing and animal size, they are the odontocete group most likely to have good low frequency sensitivity. Third, these whales are abyssal divers and are likely to have well-developed hearing which must be balanced against robustness of structure. Are both systems optimized or is hearing necessarily compromised?

This research addresses specific needs of the ONR marine mammal program by analyzing potential LF sensitive hearers, fulfills a basic need for new data on previously undescribed ears in marine mammals, and provides new and important insights into what modifications of a mammalian auditory system facilitate equilibration in deep-diving. In addition, one important but unanticipated aspect of this work is that the results are timely; they provided seminal data for the 1998 NATO panel deliberations on acoustic impacts in beaked whales and were fundamental to recent beaked whale stranding assessments.

**APPROACH:**

Whole heads and ears were obtained as post-mortem specimens from species selected because of their known ability to perform sustained, deep dives. Specimens were examined first by computerized tomography (CT and MRI) to assess the architecture and tissue distributions of the peripheral auditory system and related regions. Measurements and three-dimensional reconstructions of the heads and ears were obtained from the scan data and images. In a subset of heads selected by condition or rarity, the entire temporal bone was extracted and preserved to allow further light microscopic examination. The data from the scans, reconstructions, and dissected tissue were compared with similar data from other marine mammal species to determine what structural specializations are present in the auditory regions of deep-diving species. A description of the ear region was provided for each species describing unique elements

found in deep-diver ears and the probable relevance to the ability of these animals to withstand large atmospheric pressures encountered in abyssal dives.

## RESULTS:

This project capitalized on several innovations in functional analytical methods for marine mammal ears that were developed under prior ONR support. Data for functional analyses were obtained using three-dimensional reconstructions of the middle and inner ear from computerized tomography coupled with traditional dissection. The 3D models show undisturbed anatomy of the inner ear labyrinths, middle ear and throat air spaces, ossicular chain structure, and specialized airway bony and cartilaginous supports. In both the beaked whale and elephant seal heads examined, significant differences were found in ear and airway anatomy compared to other marine and land mammals.

During the two funded research years, reconstructions and analyses of heads from 5 new species were obtained: *Ziphius cavirostris*, *Mesoplodon bidens*, *Mesoplodon densirostris*, *Mesoplodon europaeus*, and *Mirounga angustirostris*. In addition, ears blocks from Atlantic sperm whales (*Physeter catodon*) were processed. Total specimen distribution was as follows:

*Ziphius cavirostris* - 2 ears, 1 head; 1 ear sectioned.

*Mesoplodon bidens* - 3 heads; scan only.

*Mesoplodon densirostris* - 2 heads, scans only

*Mesoplodon europaeus* - 1 head; scans only

*Mirounga angustirostris* - 5 heads, 1 ear sectioned

*Physeter catodon* - 4 ears, 1 sectioned

CT and MRI data are complete for all species and specimens. Based on available resources, only one ear for each major division was processed in celloidin and stained.

CT and gross measurements of interaural distances in elephant seals provided new interaural time difference (IATD) measurements and the first calculation of an IATD function specific to major pinniped groups. The IATD values for both male and female elephant seals suggest that these animals have an IATD most consistent with canal based reception under water. This is similar to results in harbor seals but contrasts sharply with that of the sea lion which appears to be primarily air adapted. Elephant seals have exceptionally broad apical basilar membranes (800  $\mu$ ), consistent with good low frequency hearing but also narrow basal membranes (100 micron) suggesting some ultrasonic ability. The ossicles are distinctive and include a club-like stapes that resembles the closed stapes of some odontocetes. Ganglion cell counts for the ear sectioned were 49,539 with a membrane length of 45.7 mm for an average density of 1084 cells/mm.

Analyses of sperm whale ears were limited because of poor tissue quality. The basilar membrane was missing in the sectioned material, however ganglion cells remained as did the major bony features. The sperm whale inner ear is typically odontoid, having a substantial outer lamina for ~57% of the cochlear canal which presumably supports and stiffens the basilar membrane, providing ultrasonic hearing. Ganglion cell counts for this specimen totaled 161,678 with a surprisingly high density of 2,900 cells/mm despite poor preservation.

The beaked whale scans revealed the most exceptional anatomy of all groups. Beaked whales have a fundamentally odontoid temporal bone but there is an auxiliary bony element at the Eustachian tube aperture. This sesamoid shaped bone attaches to the tube and appears to act as a strut to prevent closure. The periotic is also partly fixed to the squamosal, like that of the physeterids but less substantially than in mysticetes. The inner ear is classically odontoid with one notable exception: the vestibular divisions are remarkably well developed. The vestibule is large and bulbous and the vestibular nerve trunks appear to represent well over the conventional 5% of the VIIIth nerve common in other cetaceans. Ganglion counts are incomplete at this time.

## SIGNIFICANCE

As the first study to focus on deep diving ears, this research provided new insights into how land mammal ears were retooled to deal repeatedly and simultaneously with cold, depth, and barotrauma. The scan analyses provided new data on the auditory and cranial structure of deep-diving whales and pinnipeds and show deep divers have, collectively, specialized Eustachian tube developments, unusually well-developed vestibular elements, and derived ear canals. The data suggest that auditorially the elephant seal is the most aquatic and low frequency adapted of the pinnipeds but also suggests it has a wide hearing range. The elephant seal middle and inner ear is essentially low frequency adapted although there are ultrasonic

elements evident. Beaked whale ears are even more uniquely structured, with anomalously well-developed vestibular elements and heavily reinforced Eustachian tubes. Like other odontocetes, sperm whales and beaked whales have inner ears that appear to be primarily ultra-sonic adapted. However, the unusual level of vestibular system development and large bore, strutted Eustachian tubes that characterize beaked whales may impart special resonances and acoustic sensitivities..

#### WORK PLAN:

The short term goals of this project were completed, however it raised several issues that warrant further investigation, particularly hearing range, sound reception, and head resonance modeling. The whole head specimens obtained in this study have been properly preserved and it is feasible to obtain additional material through the NMFS network. More comprehensive light and electron microscopy data on these ears would allow us to determine their hearing ranges and to analyze the sub-structure and conformation of vestibular, middle, and inner ear complexes. More important, they would allow the necessary measurements to determine cranial and Eustachian tube resonances. Given the most recent developments in imaging and segmentation, it is now feasible to consider whole head FEM analyses that would provide high confidence functional simulations of specific frequency range responses in these animals.

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